XLII.—*On the Fecundation of the* Coniferæ.

By Dr. W. Hofmeister*.

Robert Brown's prophecy, that the Coniferæ would furnish the fittest material for the detection of the act of reproduction in Phanerogamic plants, on account of the length of time occupied in the development of their seeds, has not been fulfilled. Long as the period is in the Coniferæ, especially in the Firs, between flowering and maturity, the development does not progress gradually, but in intermittent leaps; in the most decisive epochs it advances with great rapidity, and no external sign is given of this. Difficulties are placed in the way of observation, over and above those depending upon the structural conditions, which explain sufficiently not only the differences of opinion of the many investigators who have busied themselves with this question, but also the fact that a by no means inessential feature of the process of development has hitherto escaped observation.

Schacht has lately published some remarks upon this subject†. The conclusions he draws from his latest observations, which deviate from the account given by me three years ago, are essentially as follows:—

"Active filaments (spermatozoids) *are not formed at any time* in the pollen-tube of the Coniferæ I have examined. The act of impregnation in the Coniferæ *is in no way* comparable with the formation of the germ of the higher Cryptogamia in the interior of the 'germ-organ' [*Keim-organ*; this is the name applied by Schacht to the archegonium].

"*No free cells* originate in the *corpuscula* of the Coniferæ. What Hofmeister regarded as cells, are globular spaces filled with clear fluid, bounded by a denser substance containing fine granules; they are what are called pseudo-cells (vacuoles) not uncommonly occurring elsewhere, and making their appearance in the same way even in the pollen-tube of the Coniferæ themselves * * *. From this it is clear that Hofmeister's view, that a cell existing in the *corpusculum* is impregnated by the pollen-tube, *rests upon a misconception*, since *no* free cell exists in the *corpusculum* before the entrance of the pollen-tube (p. 288).

"When the pollen-tube has penetrated into the *corpusculum* in the Scotch Fir (*Pinus sylvestris*), it soon swells up slightly; it displays itself as a small vesicle in the apex of the *corpusculum*. By the formation of a horizontal septum a cell originates in this vesicle; this cell divides cross-ways into four daughter-cells. To

* From the 'Flora,' Sept. 14th, 1854. Translated by Arthur Henfrey, F.R.S. &c.

† Beiträge zur Anatomie und Physiologie der Gewächse, Berlin, 1854, pp. 287 and 324.

the short sac-like part of the pollen-tube hangs, at this time, in the summit of the *corpusculum*, the body composed of four cells, which is soon afterwards to be found at the bottom of the *corpusculum*, that is to say, at the part opposite to the point where the pollen-tube entered. This cellular body, out of which are formed not only the so-called 'lower rosette,' but the suspensors and the first cell of the embryo, makes its way very gradually to the bottom of the *corpusculum*, detaching itself from the sac-like portion of the pollen-tube. For the contents of the *corpusculum* become very gradually more limpid from above downwards; the lower, denser portion, still filled with vacuoles, consequently supports the cellular body, till at length it reaches its goal and then undergoes further development. Not unfrequently, however, a division of the four cells in a horizontal direction takes place earlier, on which account the cellular body situated at the base of the *corpusculum* ordinarily consists no longer of four *simple* cells arranged side by side in a rosette, but of four double cells. I traced this cellular body both in its formation from the end of the penetrating pollen-tube, and also in its course downwards, so that I have seen it perfectly developed, composed of four cells, still connected with the sac-like portion of the pollen-tube, in the apex of the corpusculum, then in the middle of the latter, and finally in the place of its destination, the base of the corpusculum (p. 326).

"In the Yew (*Taxus baccata*) it is well known that the pollen-tube applies itself as a large vesicle over the summit of the embryo-sac; it descends into the excavations under which the *corpuscula* lie, and forms even there, before it has broken through the softened wall of a *corpusculum*, in its interior (and apparently by division, not by free cell-formation) a body composed of four cells arranged like a rosette, which probably originates, like the body composed of four similar cells on the pollen-tube of *Pinus*, by the division of *one* mother-cell (whether by a single or two successive divisions is a question). I was fortunate enough *several times* to detach uninjured the pollen-tube overlying a *corpusculum*, so that I was enabled to examine the cellular body lying in a pouch-like protrusion of the pollen-tube, most closely, *several times* and *on all sides*. The apex, or rather the pouch of the vesicularly expanded pollen-tube, in which the said cellular body lies, in the next place sinks into the *corpusculum*, and gradually fills it up by expanding until its size corresponds to the cavity of the *corpusculum*. The cellular body situated in the pouch of the pollen-tube, now inside the *corpusculum*, enlarges meanwhile by repeated, but not always perfectly regular cell-division * * *. I succeeded in dissecting out the portion of the pollen-tube which had penetrated into the *corpusculum*, *perfect*,

in various states of development. Ordinarily however the pollen-tube is torn off at the point where it fits the opening of the *corpusculum*."

It will be perceived that Schacht's present description removes two of the most important points of difference existing between him and me. The untenable assertion, that in the Abietineæ the pollen-tube gradually fills up the *corpusculum*, and then produces the first cell of the embryo in its interior, is wholly retracted. The young pro-embryo of *Taxus* is represented, in accordance with nature, as composed of parallel longitudinal rows of cells, not drawn as formerly*, as a longish-ovate mass of twelve-sided cells. The microscopical figures, the interpretation of which is now in question, do not differ importantly from what I have seen.

In recent years, my researches have been almost exclusively directed to the fecundation of the Coniferæ, in those few weeks during which the most decisive stages of development are passed through. I have especially endeavoured to make out the course of development of those free cells which make their appearance in the expanding pollen-tube, to which I directed attention on a former occasion†. I have not yet arrived at a decision on this point; but on the other hand, I have complete observations which make me regard the new views of Schacht as unfounded. I publish the existing results of my later researches, in the hope of stimulating some other observers to form an opinion on this subject from their own observations, in the course of the next few years. First of all, however, I will call attention to the two facts, sufficiently established before, which stand in direct opposition to Schacht's present account, viz. the presence of numerous free cells in the *corpusculum* before the arrival of the pollen-tube, and the circumstance that the first cell of the pro-embryo may be observed, in all the three great divisions of the Coniferæ, as a *simple* cell (not originally as a rosette of cells) pressed into the lower concavity of the *corpusculum*‡.

It is quite inexplicable how Schacht can question the first of these conditions. The majority of observers, Mirbel and Spach, Gottsche and Pineau, mention these cells; Pineau has figured them in *Pinus sylvestris*. The vacuoles, which appear earlier than in the fluid contents of the *corpuscula*, and which Schacht describes quite correctly, have nothing in common with these cells.

In the Abietineæ, as in the Cupressineæ, each *corpusculum*

* Schacht, Entw. des Pflanzen-Embryon, t. 9. figs. 11, 13.

† Vergleichende Untersuchungen, &c., p. 132 (1851).

‡ *Ibid.* pp. 133, 136, 137. This stage of development, and still more the preceding, of which I shall speak subsequently, are passed through so rapidly, that their being overlooked is readily excusable.

originally contains only one very large central vacuole; the protoplasm of the contents of the *corpusculum* forms a layer over the wall, on which the primary nucleus of the cell is imbedded*. This condition is at an end early in the Abietineæ, in *Pinus sylvestris* at the beginning of June. The watery fluid which filled the vacuoles becomes distributed into the continually multiplying smaller globular cavities of the protoplasm, the dimensions of which become progressively smaller in proportion to the total internal cavity of the still constantly enlarging *corpusculum*. The free globular cells now make their appearance outside these vacuoles, which from this time disappear one after another. This condition is particularly evident in *Pinus canadensis*, where one or two of these vacuoles still exist shortly before the impregnation, among the collection of floating cells which fill up the whole *corpusculum*. That the said structures are cells is proved, not only by their aspect and the presence of a nucleus, but more particularly by their often-observed multiplication†.

In the Cupressineæ the one central vacuole, and also the primary nucleus of each *corpusculum*, are retained until a short time before the impregnation. A few days only (the space of time cannot be stated accurately on account of the seeds of the Cupressineæ not being developed simultaneously; I have every reason to believe it is at most forty-eight hours) before the pollen-tubes reach the upper ends of the *corpuscula*, by pushing aside the rosettes of cells covering them, the single vacuole becomes broken up into several, between and especially above which the formation of free spherical cells takes place. Meanwhile the primary nucleus of the *corpusculum* slowly disappears; its nucleoli remain visible longer than its membrane.

In general, among the numerous daughter-cells of the *corpuscula*, those situated in the upper end, next the micropyle, are farther developed than the rest. While the latter appear destitute of a membrane, as 'primordial cells' (naked primordial utricles), the former are usually enclosed in a demonstrable cellulose coat; in the Firs (*Pinus sylvestris, austriaca, maritima*) often appressed against the wall of the *corpusculum* or impressed into its upper convexity, like the germinal vesicles of the Monocotyledons and Dicotyledons; in the Pines (*Pinus Picea*, L., and *canadensis*) mostly swimming free, not globular however here, but ovate. In *Taxus*, where the number of comparatively large cells appearing free in the *corpusculum* is but small, a single one ordinarily swims free in the centre of the *corpusculum*, while the remainder adhere to various points on the wall. Here, there is not the

* *Ibid.* t. 28. figs. 4, 5.

† Vide Pineau, Ann. des Sc. Nat. 3 sér. xi. pl. 6; and my Vergleich. Untersuch. pl. 29. figs. 1, 3–5.

least difficulty in observing these cells; in the Abietineæ and the Cupressineæ, on the contrary, their outlines are often difficult to detect, on account of the fluid contents of the *corpusculum* and its daughter-cells refracting light in exactly the same degree. But by keeping the preparation for a longer time in water, still better by treating it with a very dilute acid, all the conditions come out clearly even in these.

The internal walls of the upper part of the *corpuscula* of the Cupressineæ become thickened, often very strongly, in the manner of scalariform vessels. In *Biotia orientalis*, in particular, these walls when viewed in face present a very elegant appearance, through the transverse streaking, depending on the small breadth and close apposition of the slit-like thinner parts of the membrane.

The formation of the free cells in the interior of the pollen-tube does not commence until its arrival at the albumen-mass, in the Cupressineæ (*Juniperus communis*, *Sabina* and *virginiana*; *Biotia orientalis*), after the rupture and passage through the membrane of the embryo-sac (dissolved at the point of contact), and after the hollow above the apex of the *corpusculum* has been filled up. The earliest conditions (recognized as early by the *corpuscula*, with untouched rosette lids, still containing a large central vacuole, the formation of free cells in their interior not having yet taken place) display, swimming in the centre of the expanded portion of the pollen-tube, *one* large, free, globular cell with a very soft membrane, through the fluid contents of which, rendered opake by numerous granules, a central globular nucleus shines as a lighter space. Preparations in which the pollen-tube had commenced the displacement of the rosette of cells covering the *corpuscula*, frequently exhibited *two* such cells, or the cell had a longish ellipsoidal form and contained two nuclei, one in each focus. In a few cases I saw three, in two cases four such globular cells in the end of the pollen-tube. When there are more than one, they are always smaller than a solitary one. Other pollen-tubes, taken from ovules where the *corpuscula* were filled with daughter-cells, contained in place of one or more of these large cells, one or two groups of four to eight smaller cells, with similar contents to the larger, adherent to the wall. Sometimes a number of such smaller cells occur with one large one in the same pollen-tube. In many cases the smaller separate from one another when the preparation is pulled about in extracting the pollen-tube. When these smaller cells are burst by pressure, three things may be distinguished in the contents: very small granules, globular or angular, in which the three dimensions are about equal; longish corpuscles, spindle-shaped or stick-shaped, coloured brown by iodine, like the

smaller granules; lastly, minute vesicles with very finely granular, almost transparent contents, of diameter about equalling the length of the spindle-shaped bodies.

It appears indispensable to the accomplishment of the impregnation, that the pollen-tube should, in the Cupressineæ also, completely displace or push aside the covering rosette of the *corpusculam*, so as to come into direct contact with the upper convexity of the *corpusculum*. My former statement*, contrary to this, seems to depend upon an erroneous interpretation of an observation: either the large cells, apparently situated in the lower concavity of two *corpuscula*, observed then (only in one case) with a perfect preservation of the rosette of cells closing up the corpuscula, may have been abnormally enlarged sister-cells of these *corpuscula*, beside, not in the *corpuscula*;—or, the pollen-tube may have reached the impregnated *corpusculum* laterally, by a protruded process removed in making the section. In an uncommonly large number of examinations, I have never met with anything similar in *Juniperus*. Cases corresponding to the first hypothesis, I have observed repeatedly, as before †, in *Cupressus*, in which the development of pollen-tubes and of embryos was wholly arrested every year, while the albumen and the *corpuscula* attained to their full size—as ordinarily happens in our climate, probably resulting from the low temperature at the period of the discharge of the pollen. Phænomena analogous to the second conjecture occur in the Abietineæ, where it happens at times, in the most varied species, that the pollen-tube makes its way to the *corpusculum* by penetrating laterally through the tissue of the albumen, and not by the appointed way, through the covering-cells of the *corpuscula*. In *Biotia orientalis* also I have observed a pollen-tube penetrate into the endosperm far to one side of the group of *corpuscula*; it contained one of the often-mentioned large cells.

Ordinarily the pollen-tube sends out a short process into the *corpuscula* to be impregnated, pushing inwards the softened, apparently thicker membrane of their upper convexity. At the same time it pushes the remains of the compressed covering-cells before it, and penetrates into the interior of the *corpusculum*, through that gelatinous layer, if this has not been, as often happens, already dissolved and destroyed. More rarely the pollen-tube merely rests upon the summit of the *corpusculum*; it is equally rare for it to penetrate into it more than about $\frac{1}{10}$th of its length. Once I found it (in *Juniperus communis*) advanced as far as $\frac{1}{8}$th of the longest diameter of a *cor-*

* Vergleich. Unters. p. 131. pl. 33. fig. 12.
† *Ibid.* pl. 33. fig. 26.

pusculum. It had only just touched the *corpusculum* immediately adjoining this one, but impregnation had taken place in both. The rarest cases are those in which the pollen-tube sends out a slender, very short pouch between the covering-cells of the *corpusculum*, these at the same time retaining pretty much their original position and form.

I always found the projecting pouches of the pollen-tubes closed, even in the cases where it was evident impregnation had but just taken place: when the first cell of the pro-embryo, free and of spherical shape, had not yet descended to the bottom of the corpusculum. In *Biotia orientalis* it is not difficult to prepare the pollen-tube free from the albumen, and to extract its perfectly closed projection out of the impregnated *corpusculum*. In the Junipers this manipulation is rendered difficult, by the rapid expansion of the contents of the corpuscula both in pure water and in solutions of salts or sugar. But the phænomena usually occurring here in the expansion and final bursting of the *corpuscula* are so much the more convincing; the pouch of the pollen-tube which had penetrated into the *corpusculum* is turned inside out, and a rapid whirling current is set up into it. Finally, the vesicular expansion bursts, and the contents of the *corpusculum* make their way into the interior of the pollen-tube.

After the arrival of the pollen-tube at the *corpusculum*, one of the cells which had been produced in its interior increases in circumference and in its finely-granular contents. In none of the Cupressineæ did I see this cell in contact with the pouch of the pollen-tube which had penetrated the *corpusculum*. On the contrary, in many cases the position of its unaltered sister-cells rendered such a process in a high degree improbable, inasmuch as these so filled up the space between that cell and the end of the pollen-tube, that the enlarged cell could not have made its way through them. In the earliest conditions of which I could get a view, the enlarged impregnated cell was in the upper third of the *corpusculum*; in other cases in its centre or lower end, where it is at first only loosely imbedded. In many instances a globular nucleus without nucleoli may be detected in its centre, in other cases this cannot be made visible. The region of the *corpusculum* below the descending impregnated germinal vesicle, is remarkably poor in cells.

After the impregnated germinal vesicle has become firmly adherent to the lower end of the *corpusculum*, it divides, in all cases, by a horizontal septum. Then first, often only in the lower cell of the two, occurs the formation of longitudinal septa, converting the pro-embryo into a body composed of parallel longitudinal rows of cells.

During this process, those small cells with opake contents

adherent to the wall, are ordinarily to be found in the interior of the pollen-tube, but now separate from one another. Some have burst; the solid bodies contained within them are diffused in the cavity of the pollen-tube. Many of the still intact cells are also frequently shrivelled up. Sometimes the pollen-tube contains no cellular structures of any kind during the impregnation; but I never missed then the spindle-shaped and stick-shaped bodies which were especially accumulated in the pouches which penetrated into the *corpuscula*, being often joined in numbers into bundles. Pollen-tubes of this character could be more readily dissected out free than those of the first kind, both in *Biotia* and in *Juniperus*. Lastly, it happens that those large cells are preserved, mostly the majority, in the pollen-tube, during and after the impregnation; but very much changed; flattened into a lenticular or meniscus form, firmly appressed to the wall of the pollen-tube, sometimes at the side, sometimes at the bottom. The central nucleus has now vanished; in its place are perceived in the cloudy (by transmitted light, yellowish) contents of the cell, a definite number (8—16) of sharply circumscribed, circular, bright places (nuclei), between which run delicate reticulated lines (the faces of contact of daughter-cells) only to be detected with the best defining magnifiers. This is the appearance in face; in profile it is perceived that it is a simple layer of prismatic cellules, into which the large cell has been divided. In a single instance a fresh, freely swimming, globular, large cell with a central nucleus was observed in the pollen-tube of an ovule of *Biotia orientalis*, in two of the *corpuscula* of which the pro-embryos had already made their appearance. In all these cases those stick-shaped bodies were to be found, even though sparingly, in the pollen-tubes, outside the large cells. In pollen-tubes in this condition (which effect impregnation as freely as the others) I never met with the protruded pouches sent into the *corpuscula*. There is not the slightest difficulty here in separating the pollen-tube, uninjured, from the albumen and the fertilized *corpuscula*.

The appearance of free globular cells in the widely expanded end of the pollen-tube of *Taxus baccata*, formerly described and figured by me*, is also in this plant followed by alterations of these cells similar to those occurring in the *Cupressineæ*. In farther developed ovules, such cells appear firmly applied against the wall of the pollen-tube, much flattened down, and divided by walls standing crosswise and perpendicular to the membrane of the pollen-tube. This condition of the said cells, the size of which mostly exceeds that of the young pro-embryo,

* Vergleichende Unters. p. 132, t. 31. fig. 18.

strikingly resembles in the profile view young conditions of the pro-embryos, of the *Abietineæ* more particularly, in a lesser degree those of *Taxus* itself.

I found the behaviour of the pollen-tubes to the impregnated *corpusculum* twofold, in *Taxus* as in *Juniperus*: either the pollen-tube sends a short process into the narrow mouth of the *corpusculum*, or it applies itself broadly over the summit of the *corpusculum*, with a shallow convexity of its membrane projecting a little way into it. In both cases the five or six cells of the covering-rosette of the *corpusculum* were mostly only pushed asunder and squeezed flat, not completely absorbed. The pollen-tube was very firmly adherent to them. As they are very intimately connected with their neighbouring cells, the pollen-tube is usually torn at this place in the attempt to dissect it out from the impregnated *corpusculum*. In rare cases the adhering cover-cells of the *corpusculum* are separated from their connection and lifted up with the pollen-tube. Then even the outline of the pouch of the pollen-tube is frequently rendered indistinct by adherent and contained granular mucilage, so that it is not often to be clearly made out whether this pouch is closed or has a minute opening. But so much the more certainly can we be convinced of the closure of the *flat* protusions of the pollen-tube squeezed in between the cells of the covering-rosette; the form in which, as it appears to me, the impregnation most frequently takes place.

According to Schacht's idea, one of these cell-rosettes, similar to young pro-embryos, must be formed in the pollen-tube over each *corpusculum* to be impregnated, into which, then, after the destruction of the cover of the *corpusculum*, the large portion of the pollen-tube lying above it has to bulge out and insert itself, carrying the four- or many-celled pro-embryo to the bottom of the *corpusculum*. Against this view speak not only the above-stated, but the following reasons:—

1. In all cases, I find the cell-rosette, which makes its appearance in the pollen-tube, somewhat larger than the earliest rudiment of the pro-embryo, and much too large to pass through the ordinarily narrow mouth of the *corpusculum*. In particular, the part of the pollen-tube which is torn off in the attempt to extract it from the just-impregnated *corpusculum*, is very small, quite out of proportion to the size of the cell-rosettes in the pollen-tube and of the young pro-embryo.

I give a few of the measurements:—

a. Transverse diameter of the young, four-celled pro-embryo, 0·753 millim.

Transverse diameter of the mouth of the *corpusculum* into which a short protrusion of the pollen-tube had penetrated, 0·111 millim.

b. Smallest diameter of a cell-rosette adherent to the lateral wall of the pollen-tube, 0·500 millim.

Greatest diameter of the same, 1·225 millim.

Transverse diameter of the mouth of a *corpusculum* impregnated by the same pollen-tube, 0·124 millim.

Transverse diameter of the unicellular rudiment of a pro-embryo situated at the bottom of the same *corpusculum*, 0·832 millim.

c. Transverse diameter of the orifice of a pollen-tube torn off at the point of entrance into the *corpusculum*, 0·013 millim.

Upper end of the eight-celled pro-embryo situated at the bottom of this *corpusculum*, 0·861 millim.

2. In a pollen-tube dissected free from an impregnated *corpusculum*, I detected the shrivelled remains of the cell-rosette contained in it, situated above the point where the latter had been torn off.

3. The upper part of the *corpusculum*, not filled up by the pro-embryo, contained free cells exactly similar to those existing there before impregnation.

4. There is no reason to doubt that the *simple* cells, frequently observed, filling up the lower concavity of the *corpuscula* of *Taxus baccata* and *canadensis*, are the primary cells of the pro-embryos. But, in that case, they could not derive their origin from the cell-rosettes contained in the pollen-tube.

I owe to the kindness of my friend Schacht, a sight of his preparations of *Taxus baccata*. They have not been sufficient to convince me of the correctness of his interpretation. Schacht has several times observed that in *Taxus* also (as frequently happens in *Juniperus* and the *Abietineæ*) the pollen-tube penetrates pretty deeply into the *corpusculum* and there expands to a certain extent;—perhaps an individual peculiarity of the specimens which furnished Schacht with the materials for his investigations; throughout very numerous examinations I have never met with anything of the kind. These vesicularly-expanded pouches of the pollen-tube, separated from the *corpuscles*, were of very various magnitudes. I estimated the diameter of one at 0·1 millim.; of another at 0·4 millim. The largest would therefore have about half filled a *corpusculum*; a condition which is sometimes at least approximatively attained even in the gigantic *corpuscula* of the *Abietineæ*. The but indistinctly perceptible cellular structures existing in these protruded sacs

of the pollen-tubes bore no resemblance whatever either to young pro-embryos of *Taxus*, or the cell-rosettes originating together with them in the pollen-tubes, of which latter Schacht possessed very elegant preparations.

There cannot be the slightest doubt that the pro-embryo of the *Abietineæ* may be observed as a *simple* cell impressed into the lower concavity of the *corpusculum*; the first division of this cell taking place, as in *Juniperus*, by a transverse wall. Very often, the crosswise arranged longitudinal walls, which lay the foundation of the construction of the pro-embryo out of four longitudinal rows of cells, make their appearance only in the lower of the two cells; or the upper and larger of the two is divided only once by a longitudinal wall. These cases, frequent in *Pinus Strobus* and *canadensis*, contradict Schacht's view most decidedly. Among the preparations which Schacht was good enough to show me, I found one which exhibited a free cell-rosette of four tough-walled cells in the upper concavity of a *corpusculum* which had been halved by the section. In another *corpusculum* of the same albumen, opened by the same cut, it could be made out that the pro-embryo which had originated there had already sent out embryonal tubes $\frac{1}{4}$ of a line long.

Whoever has made investigations on the fertilization of the Abietineæ, knows how surprisingly simultaneous, in all ovules of the same species, is the passage through the exceedingly rapid course of the first stages of development of the pro-embryo. I may mention by way of example, that at Leipsic, in the present year (1854), not a pollen-tube had penetrated to a *corpusculum* on the 22nd of June, while only three days later, on the 25th of June, among several hundreds of impregnated *corpuscula* that were examined, not one could be found which did not contain at least one 4-celled pro-embryo. I regard it as quite impossible that one *corpusculum* can outstrip its neighbour by some twelve days, in one and the same albumen, in the development of the embryo. I look upon that preparation of Schacht's as an artificial product; I believe that cell-rosette—perhaps the end-cells of the embryonal tubes of the neighbouring *corpusculum*—must have been carried, in slicing, into the impregnated *corpusculum*. Such occurrences readily happen in making sections of objects held between the thumb and finger. I possess a preparation of *Pinus canadensis*, in the opened, apparently otherwise undisturbed *corpusculum* of which, lies an epithelial cell from the skin of the finger-tip. Other preparations of Schacht's exhibited, beside the introduced end of the pollen-tube, two cells, flattened by mutual pressure, appressed against the upper concavity of the corpusculum—germinal vesicles, such as I have already figured in a similar condition in *Pinus austriaca*. Schacht has no ob-

servations to speak directly in favour of his assertion that the said cell-rosette is formed from the pollen-tube by "tying-off" (*abschnürung*).

My later researches, made principally upon *Pinus canadensis*, have led me to the conclusion that the appearance of the first cell of the pro-embryo in the bottom of the *corpusculum* is a secondary condition. Many times, in *corpuscula* into or upon which pollen-tubes had penetrated, I detected in the middle of the *corpusculum* free, ovate cells, distinguished above their neighbours—unimpregnated germinal vesicles—by size, and above all by the extremely abundant granular mucilage they contained, in the same way as is the case in the impregnated germinal vesicles of *Biotia* and *Juniperus*. That these cells gradually make their way to the bottom of the *corpuscula* is rendered still more probable, since the undoubtedly unicellular forms of the pro-embryo of *Pinus canadensis* are made to draw back far away from the lower concavity of the *corpusculum*, by the application of reagents contracting the primordial utricle; a certain proof that they at first lie loose here. Hitherto I have not observed these cells—in my opinion the impregnated germinal vesicles—in immediate contact with the pollen-tube. In the cases where they were nearest to it, they were always at a distance at least equalling the longitudinal diameter of the cell. But it is nevertheless probable, that there is always direct contact in the impregnation. This would explain the coincident observations of Scheljesnow * and Cienkowski †, according to which, in *Larix europæa* (which I have not yet examined), a large cell hangs for a long time to the pollen-tube which has penetrated the *corpusculum*. The fecundation seems to be effected more slowly here than in the other Abietineæ.

I have little to add to my former statements regarding the cell-formation in the interior of the pollen-tube of the Abietineæ. During the impregnation I saw in its interior, with free starch, spherical cells with granular mucilaginous contents, sometimes combined in groups, and then eight in number. Not the least sign can be observed of an opening of the pollen-tube. The two modifications of its mode of penetration already mentioned as occurring in the Cupressineæ and Taxineæ, are met with also in the Abietineæ. While it frequently advances only as far as the upper concavity of the *corpusculum*, it still more frequently projects a hemispherical end a little way in, and sometimes penetrates tolerably deeply. I possess a preparation of *Pinus canadensis* in which it fills up a full third of the *corpusculum*.

* Bulletin de Moscou, 1849, p. 466.
† *Ibid.* 1853, p. 337. t. 7. fig. 13.